Antisolvent precipitation technique: a very promising approach to crystallize curcumin in presence of polyvinyl pyrrolidon for solubility and dissolution enhancement

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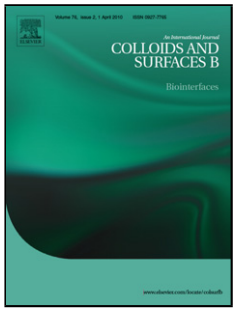

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Antisolvent precipitation technique: a very promising approach to crystallize curcumin in presence of polyvinyl pyrrolidon for solubility and dissolution enhancement

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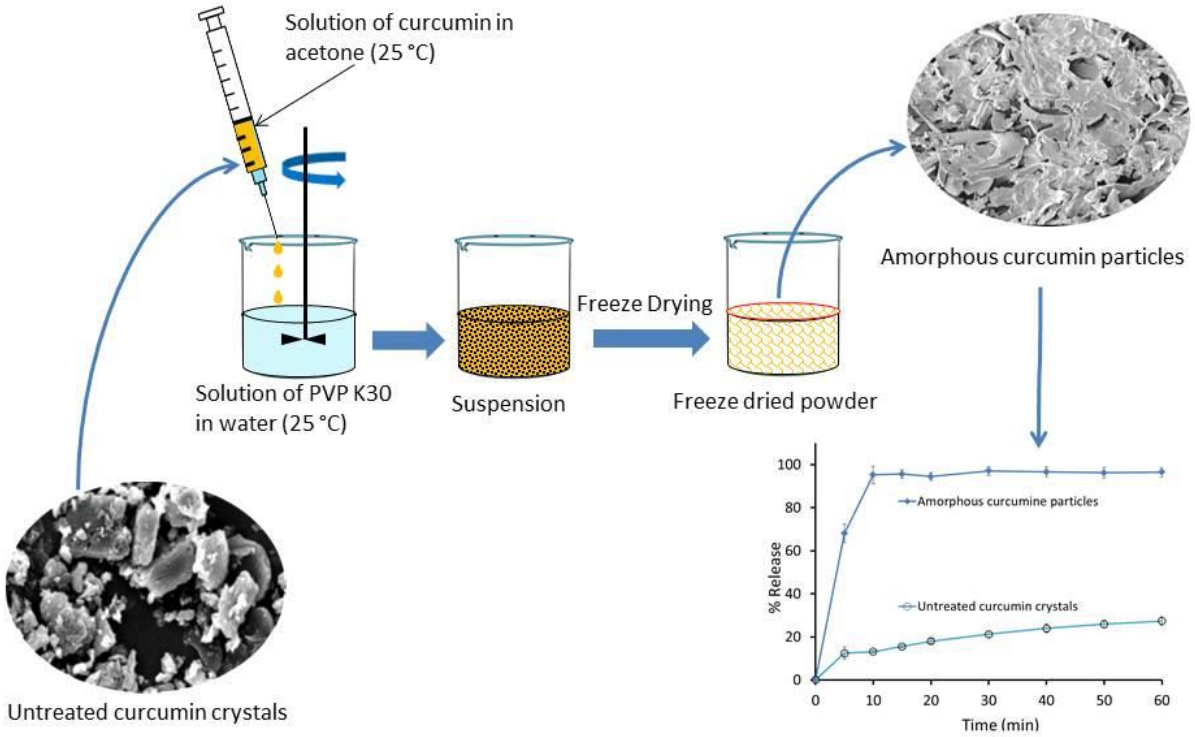
Graphical Abstract



Highlights

- Curcumin with an anticancer effect showed a poor dissolution
- Crystallization of curcumin in absence of PVP led to formation of a new polymorph
- PVP in crystallization medium led to a remarkable dissolution enhancement
- The presence of PVP converted crystalline curcumin to amorphous curcumin

Abstract

Curcumin with a vast number of pharmacological activities is a poorly water soluble drug which its oral bioavailability is profoundly limited by its dissolution or solubility in GI tract. Curcumin could be a good anticancer drug if its solubility could be increased. Therefore, the aim of the present study was to increase the dissolution rate of curcumin by employing antisolvent crystallization technique and to investigate the effect of polyvinyl pyrrolidone K30 (PVP) as colloidal particles in crystallization medium on resultant

particles. Curcumin was crystalized in the presence of different amounts of PVP by antisolvent crystallization method and their physical mixtures were prepared for comparison purposes. The samples were characterized by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray powder diffraction (XRPD) and Fourier transform infrared spectroscopy (FT-IR). The solubility and dissolution of the treated and untreated curcumin were also determined. Antisolvent crystallization of curcumin led to the formation of particles with no definite geometric shape. It was interesting to note that the DSC and XRPD studies indicated the formation of a new polymorph and less crystallinity for particles crystallized in the absence of PVP. However, the crystallized curcumin in the presence of PVP was completely amorphous. All crystalized curcumin samples showed much higher dissolution rate compared to untreated curcumin. The amount of curcumin dissolved within 10 for treated curcumin in the presence of PVP (1:1 curcumin:PVP) was 7 times higher than untreated curcumin and this enhancement in the dissolution for curcumin samples crystallized in the absence of PVP was around 5 times. Overall, the results of this study showed that antisolvent crystallization method in the absence or presence of small amounts of PVP is very efficient in increasing the dissolution rate of curcumin to achieve better efficiency for curcumin.

Keywords: Curcumin; Antisolvent Crystallization; PVP; Dissolution; Solubility; Amorphous

1. Introduction

There are lots of techniques that have been used to enhance the dissolution rate or solubility of poorly water-soluble drugs [1]. Crystal engineering or crystal manipulation has been the focus of many research activities to achieve this goal during the past few years. Crystal manipulation has been performed by either top-down or bottom-up techniques [2, 3, 4].

Top-down **procedures involve** the breaking of drug particles by milling and/or homogenization while bottom-up **methods** involve the building up particles from drug molecules via precipitation techniques. The top-down methods suffer from some disadvantages such as the need for high-energy input, long processing time, possibility of metal contamination and wide particle size distribution [5, 6]. This has led to the popularity of bottom-up methods for the purpose of crystal manipulation. In most of these techniques the main aim is to achieve small particle size in order to increase the effective surface area of particles and consequently the dissolution rate, while other objectives such as increasing solubility and wettability were also sought. Among different bottom-up methods, spray freezing into liquid and supercritical antisolvent precipitation have not found widespread use due to the complexity of the operation procedure (need very low temperature and high pressure) and also their expenses [7, 8, 9]. However antisolvent precipitation which could be

implemented at ambient temperatures and atmospheric pressure with no need for expensive equipment has widely been used in order to improve **the** dissolution rate of poorly soluble drugs. In this method supersaturation is achieved by the addition of an antisolvent to the organic solution of drug with low solubility leading to nucleation of drug and consequently precipitation of particles [10, 11, 12, 13]. In this process **the** addition of some stabilizers during the precipitation procedure could be beneficial as they could retard or prevent particle growth by covering the surface of precipitated particles in short-term. However, an immediate drying is required to prevent further growth of particles [14, 15].

Curcumin, a hydrophobic polyphenol extracted from herbal spice turmeric, is a poorly water-soluble drug. It has antioxidant, anti-inflammatory, antitumor, anti-HIV, and antimicrobial properties [16, 17, 18, 19]. Different approaches have been implemented in literature in order to address the issue of its poor dissolution rate or low solubility. The use of polymeric nanoparticles [20, 21, 22, 23], solid lipid nanoparticles [24, 25, 26], the use of micelles [27, 28, 29] and preparation of amorphous solid dispersion systems are some examples. However there are few studies regarding the use of a crystallization procedure like antisolvent precipitation in order to achieve this goal. Thorat and Dalvi described the mechanism of particle formation pathways and polymorphism of curcumin induced by ultrasound and additives during liquid antisolvent precipitation [30, 31]. They obtained loose aggregates with amorphous nature and proposed that the use of polymeric stabilizer such as PVP could prevent crystal fusion, however no attempts have been made to investigate their dissolution rates. Kakran *et al*. have prepared curcumin nanoparticles via antisolvent precipitation in absence of any stabilizer for oral administration [32]. The authors have compared two preparation methods: antisolvent precipitation with a syringe pump and evaporative precipitation of nanosuspension. Yadav and Kumar investigated the antisolvent precipitation process to prepare curcumin nanoparticles in presence of gelatin as stabilizer for parenteral administration [33]. The effect of different process variables on particle size and size distribution of curcumin during particle formation was investigated. In the present study the antisolvent precipitation procedure has been used for manipulation of curcumin particles for oral use in the presence of different concentrations of PVP as stabilizer.

2. Materials and methods

2.1 Materials

Curcumin was purchased from Hindustan Herbal Limited Company (Haryana, India), polyvinyl pyrrolidone K30 was obtained from Fluka (Switzerland) and sodium dodecyl sulfate was obtained from Merck (Germany). All other solvents and chemicals were of analytical grade.

2.2 Methods

2.2.1 Precipitation of curcumin by antisolvent crystallization

Accurate weighed amount of curcumin (1 g) was dissolved in 10 mL acetone. **Different quantities of PVP (0, 0.5, 1 and 2 g) was separately dissolved in beakers containing 100 mL distilled water.** The solution of curcumin in acetone was added dropwise (at rate of 5 mL/min) using a syringe equipped with needle gauge No. 22, into the aqueous solution of PVP while stirred at 800 rpm at 25°C. **The final ratios of curcumin: PVP in the solutions after adding the curcumin solution to PVP solution were 1:0, 1:1, 1:2 or 2:1.** The obtained suspension was immediately freezed at -80°C for 24 h and then freeze dried using Heto freeze dryer (Denmark) for 48 h. Each crystallization experiment was repeated three times.

2.2.2 Preparation of physical mixtures of curcumin and PVP K30

The physical mixtures of curcumin and PVP with the similar ratios as above were also prepared for comparison purposes. The sieved fractions (passed through 60 mesh sieve) of the curcumin **and PVP were mixed in tumbling mixer rotating at 50 rpm for 15 min. The obtained mixtures were kept in glass vials until further studies.**

2.2.3 Morphological analysis of samples

A scanning electron microscope (LEO 1450 VP, Germany) was used to investigate the morphologies of **untreated** curcumin and precipitated samples. Untreated curcumin and all freeze-dried samples were coated with a thin gold-palladium layer by sputter coater (SC 7620, England) prior to observation.

2.2.4 Solubility measurement

The solubility of **untreated** curcumin, physical mixture and crystallized samples was determined by adding an excess amount of samples (about 5 mg) to screw-capped glass vials containing 20

mL distilled water. The vials were shaken for 48 h at 25°C. The suspensions were then filtered through a 0.45 µm filter and the concentration of curcumin was determined spectrophotometrically at 426 nm (Cecile 900, USA). The test was repeated three times and the mean was reported.

2.2.5 Differential scanning calorimetry (DSC)

DSC analysis was conducted using DSC 822e (Mettler Toledo, Switzerland) equipped with a refrigerated cooling system to study the thermal behaviors of the samples. The instrument was calibrated using indium standard. 2- 3 mg samples of **untreated** curcumin and precipitated curcumin in the presence or absence of PVP were placed in aluminum pans sealed with a lid and were scanned from 20 to 200°C at a scanning rate of 10°C/min under nitrogen gas at a flow rate of 80 mL/min.

2.2.6 X-ray powder diffraction studies (XRPD)

X-ray powder diffraction patterns of the selected samples were obtained by a X-ray diffractometer (Philips, Germany). Each sample was scanned in the range of 5-60° (2θ) with a step size of 0.02° at a scan rate of 0.04/s.

2.2.7 Fourier transform infrared spectroscopy (FT-IR)

FT-IR was used to investigate any changes in the precipitated curcumin formulations at the molecular level. The spectrum was obtained using a spectrum II FT-IR spectrometer (PerkinElmer, Waltham, USA). The specimens were prepared by mixing of each sample with KBr at a ratio of 1:5 and then compressing it at a pressure of 7 tons for 2 min using a hydraulic press. The obtained discs (sample-KBr) were scanned against pure KBr disc at wavenumbers ranging from 450 to 4000 $cm^{-1}$with a resolution of 1.0 cm^{-1}.

2.2.8 Dissolution studies

A USP dissolution apparatus 2 (paddle method) (Pharmatest, Germany) was used to test the dissolution profiles of **untreated** curcumin and other samples. Each dissolution vessel was filled with 1000 mL distilled water containing 0.25% w/v sodium dodecyl sulfate (SDS) equilibrated to 37°C and the paddles rotated at 50 rpm. An accurate weight of samples equivalent to 10 mg curcumin were transferred to the dissolution medium **in powder form.** Samples were withdrawn

from the vessels at selected time intervals through filter using a syringe and were replaced with the same volume of fresh medium. The amount of dissolved curcumin was assayed at 426 nm by a spectrophotometer (Shimadzu, Japan) based on a calibration curve obtained for curcumin at this wavelength. The dissolution test of each sample was conducted in triplicate. PVP solution showed no absorption at 426 nm.

The dissolution profiles were compared by calculating the difference factor (f_1) and also the similarity factor (f_2) based on the following formulas.

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum R_t} \times 100$$

$$f_2 = 50 \times \log\left\{\left[1 + \left(\frac{1}{n}\right)\sum |R_t - T_t|^2\right]^{-0.5} \times 100\right\}$$

Where R_t is the dissolution value of the reference sample at time t and T_t is the dissolution value of the test sample at time t. Values of f_1 less than 15 and f_2 more than 50 indicate the similarity between two curves.

3. Results and discussion

Particle engineering by antisolvent precipitation is a simple and effective approach for modification of poorly water-soluble drug properties. In this study acetone was used as a solvent for curcumin and water was used as antisolvent. Among different solvents that have been used to dissolve curcumin, acetone was selected due to high solubility of curcumin in this solvent and its rapid evaporation at low temperatures. Distilled water was used as an antisolvent according to its safety, availability and low cost. PVP was also used as a stabilizer in different concentrations. Stabilizers have been utilized widely to prevent crystal growth by providing steric hindrance due to the absorption at the drug– solvent interface during particle precipitation [34, 35, 36, 37].

Upon addition of the curcumin solution to water (antisolvent), curcumin particles were formed immediately. Volume ratios of solvent to antisolvent were considered as1:10 in order to achieve small particle size. It has been reported that an increase in the proportion of antisolvent would have resulted in a decrease in particle size. Based on the classical nucleation theory, when the possibility of supersaturation is high, the lowest median particle size could be achieved [38, 39]. This phenomenon would happen because of increased rates of primary nucleation [40, 41, 42]. Higher the proportion of antisolvent, greater is the degree of supersaturation and therefore smaller is the

size of precipitated curcumin particles. The obtained suspension after addition of antisolvent was rapidly freezed in order to prevent crystal growth.

Figure1 shows the SEM images of untreated curcumin and precipitated samples in the absence or presence of PVP. As depicted in Figure 1a the untreated curcumin showed grain-like shape crystals, while the precipitated samples in absence of PVP (Figure1b) exhibited dendritic needle like crystals among shapeless particles. All samples precipitated in the presence of different ratios of PVP exhibited amorphous structure with no definite geometric shape (Figures1c, d and e) which will be discussed later in the solid state analysis of the manuscript.

These findings indicated the ability of PVP to prevent the crystal growth in the crystallization medium. Similarly it has been reported that PVP K30 could prevent crystal growth of celecoxib and resulted in formation of amorphous particles following the antisolvent crystallization [43].

Figure 1 also indicates that the increase in PVP concentration in the precipitated samples resulted in the formation of aggregated particles. This effect was probably due to the adhesive properties of PVP. Similar effect with increase in stabilizer concentration has been reported previously [33, 44, 45, 46].

Table 1 lists the solubilities of different curcumin samples. The table shows that the solubility of curcumin in the precipitated samples is significantly ($P<0.05$) higher than **untreated** curcumin or its physical mixtures with PVP which could be a reason for the better dissolution of the precipitated curcumin samples and this will be discussed extensively in the dissolution analysis of the samples.

The DSC traces of different curcumin samples and pure PVP are shown in Figure 2. The untreated curcumin showed a sharp endothermic peak at 174°C corresponding to its melting point. Pure PVP exhibited a broad peak around 80°C due to evaporation of the adsorbed water. As depicted in Figure 2 all physical mixtures of curcumin-PVP showed the endothermic peak in DSC traces which corresponded to the melting point of curcumin and this peak remained unchanged which confirmed no interaction between curcumin and PVP in physical mixtures. The precipitated sample in the absence of PVP showed two endothermic peaks at 155°C and 170°C. Two endothermic peaks at 155°C and 170°C in DSC traces of the precipitated sample in the absence of PVP indicated that the sample was partially crystalline. **The reduced intensity of the peak at 170°C and the appearance of a new peak at 155°C confirmed the reduced crystallinity of the curcumin and formation of a new polymorph during crystallization procedure according to Tharot and Dalvi** [30, 31]. They reported that raw curcumin crystals existed in monoclinic form while

curcumin precipitated by antisolvent method in the presence of ultrasound, resulted in orthorhombic form of curcumin. The melting point of this form was between 158-163°C. However, DSC results for precipitated samples in the presence of different amounts of PVP ruled out the presence of any melting transition, which was an indication of the amorphous nature of the samples. These results suggest that the crystallization procedure had a crucial role in formation of amorphous phase of curcumin and occurrence of the interaction between the drug and stabilizer. In a study performed by Kaewnopparat *et al*., it has been reported that the melting peak of curcumin has shifted towards lower temperatures in solid dispersion systems of curcumin and PVP, indicating their interaction [47]. Also the widening of the melting peak has been attributed to the presence of an amorphous phase in the solid dispersion systems.

Samples precipitated in the presence of PVP exhibited a broad peak at region 70-80°C **which could** be due to the presence of PVP in the samples, while the peak related to the melting point of curcumin could not be observed. The lack of any transition peak for curcumin indicates that the drug is in amorphous state in the precipitated samples.

The DSC traces of pure PVP, physical mixtures and precipitated samples exhibited a broad endothermic peak at region of 70-80°C which is attributed to loss of moisture content in the samples due to the presence of PVP.

The results of XRPD studies are depicted in Figure 3. The sharp peaks observed in the XRPD pattern of untreated curcumin (Figure 3a) confirmed the crystalline structure of the sample. The XRPD pattern of precipitated sample in the absence of PVP (Figure 3b) showed fewer peaks with lower intensities and also the presence of some new peaks compared to untreated curcumin. Appearance of the new peaks in XRPD patterns is probably indicating the formation of new polymorphs [31]. These results are completely in agreement with the results of DSC studies which indicated the less crystallinity for the precipitated samples obtained in the absence of PVP and formation of a new polymorph **of curcumin**. In contrast, the XRPD patterns of the precipitated samples in the presence of PVP (ratios of drug:PVP 1:1 and 1:2) (Figures 3c and 3d) showed a halo pattern without diffraction peaks, indicating that curcumin is completely amorphous which again were in agreement with the results of DSC studies. It could be concluded from these results that curcumin turned into completely amorphous state when precipitated in the presence of PVP by antisolvent crystallization procedure whilst in absence of PVP the crystallinity of the samples

has been reduced and a new polymorph of curcumin was formed. **These results are in accordance to findings of other studies on solid dispersion systems of curcumin and PVP** [47, 48].

The FT-IR spectra of untreated curcumin (Figure 4b) showed the characteristic peaks at 1626 cm^{-1} due to the stretching of C=O group, at 1508 cm^{-1} due to aromatic C=C stretching, at 1428 cm^{-1} due to phenolic C–O stretching and at 1427 cm^{-1} due to enolic C–O stretching. The presence of these major peaks in curcumin samples crystallized either in the absence or presence of PVP ruled out any changes in chemical structure of curcumin. **In addition**, a sharp peak **is** observed at 3505 cm^{-1} in untreated curcumin spectrum (Figure 4b) which is attributed to OH stretching [49]. In precipitated samples in the absence of PVP this peak **is** almost broadened and shifted to lower wavenumber at 3393 cm^{-1} (Figure 4c) suggesting a difference in the molecular environment of the hydroxyl groups relative to untreated curcumin. This peak has been widely broadened in curcumin samples crystallized in the presence of PVP (Figure 4d) indicating an interaction such as the intermolecular hydrogen bonding between curcumin and PVP. This interaction probably account for changes in curcumin crystalline structure to amorphous form. Similar results have been reported by Li *et al* [50].

The results of dissolution studies are shown in Figure 5. This figure shows that the untreated curcumin dissolves very slowly so that only 25% of curcumin was dissolved after 60 min. The addition of PVP in physical mixtures did not affect the dissolution rate of curcumin considerably (f_1=18, f_2=67). However crystallized curcumin in the absence or presence of PVP showed higher dissolution rate than untreated curcumin (f_1=73, f_2=14). As the solubility of these samples were not considerably different from **the** untreated curcumin (Table 1), the most probable reason for the increase in dissolution rate could be the formation of partially amorphous structure.

Figure 5 also shows that the precipitation of curcumin in the presence of PVP for the ratio of drug:PVP 1:1 produced the highest dissolution. The percentages of curcumin dissolved within 10 minutes of the dissolution test for untreated curcumin, for crystallized samples at 1:1 curcumin:PVP ratio and for samples crystallized in absence of PVP were 13, 90 and 70% respectively. Similar results were obtained for the effect of PVP on dissolution of curcumin and acetaminophen [5, 51]. However the increase in PVP concentration beyond the 1:1 ratio (e.g. 1:2 ratio of drug:PVP) decreased the dissolution rate of curcumin. The lack of difference between dissolution rate of samples prepared from physical mixtures of curcumin and PVP with untreated curcumin suggests that the presence of PVP by itself did not have any profound effect on

dissolution rate of curcumin, therefore the effect of increase in wettability of the particles in presence of PVP on their dissolution rate could be ruled out. As shown in Table 1 the solubility of the sample precipitated at 1:2 curcumin: PVP ratio was highest whilst the dissolution rate of this sample was the lowest compared to the other samples precipitated in the presence of PVP. Therefore considering the results of dissolution studies of the samples precipitated in the presence of different amounts of PVP again one would say that the most probable reason for the increased in the dissolution rate of precipitated samples in the presence of PVP is the formation of amorphous particles. Decrease in dissolution rate with increasing PVP concentration in **samples precipitated in the presence of PVP (curcumin: PVP 1:2) could be related to increases in the viscosity of particle microenvironment.**

4. Conclusions

The antisolvent precipitation procedure using acetone as solvent, water as antisolvent and low amounts of PVP as stabilizer is a very promising and effective method to increase the dissolution rate of curcumin. The method is simple and practical and uses safe materials. The curcumin particles obtained in this method were completely amorphous and remarkably showed higher dissolution rate compared to untreated curcumin.

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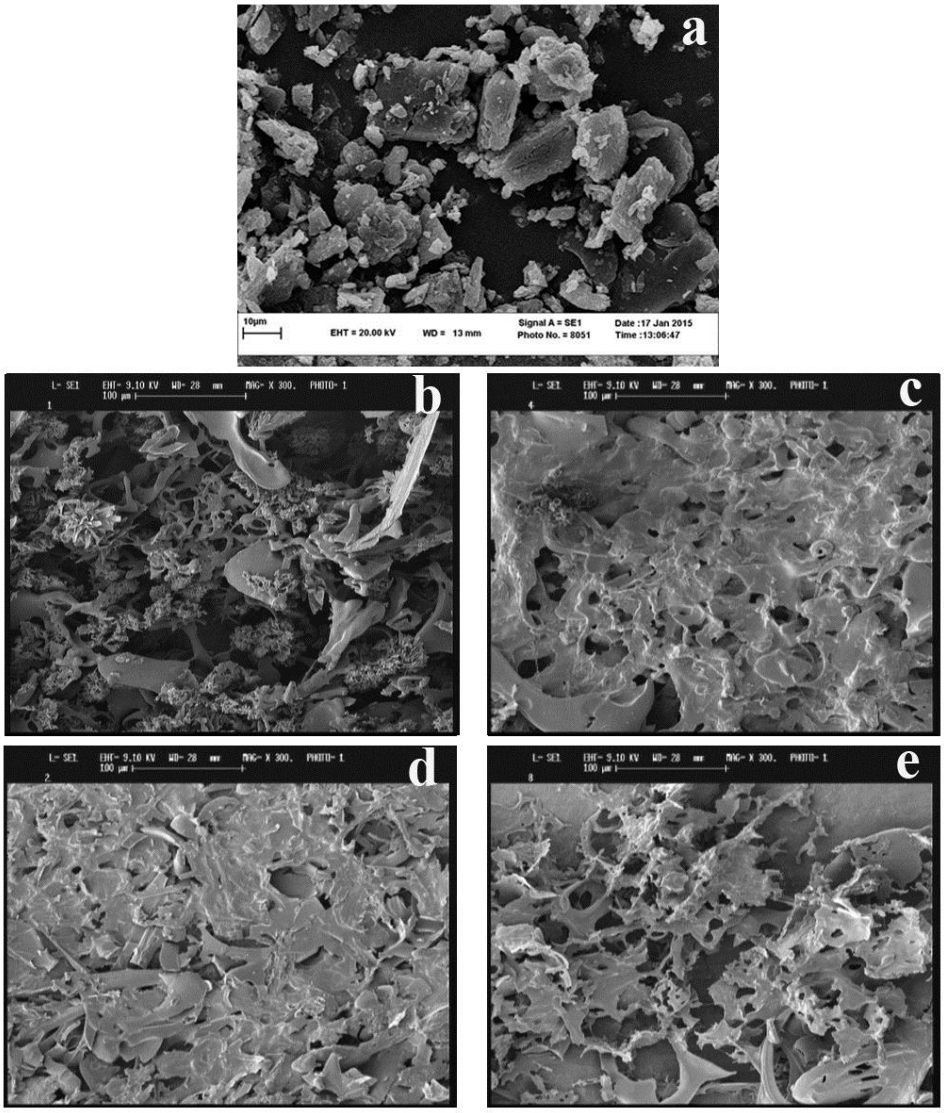

Figure 1. Scanning electron micrographs of untreated curcumin (a), precipitated curcumin samples in absence of PVP (b), in presence of PVP at 2:1 (c), at 1:1 (d), and at 1:2 (e).

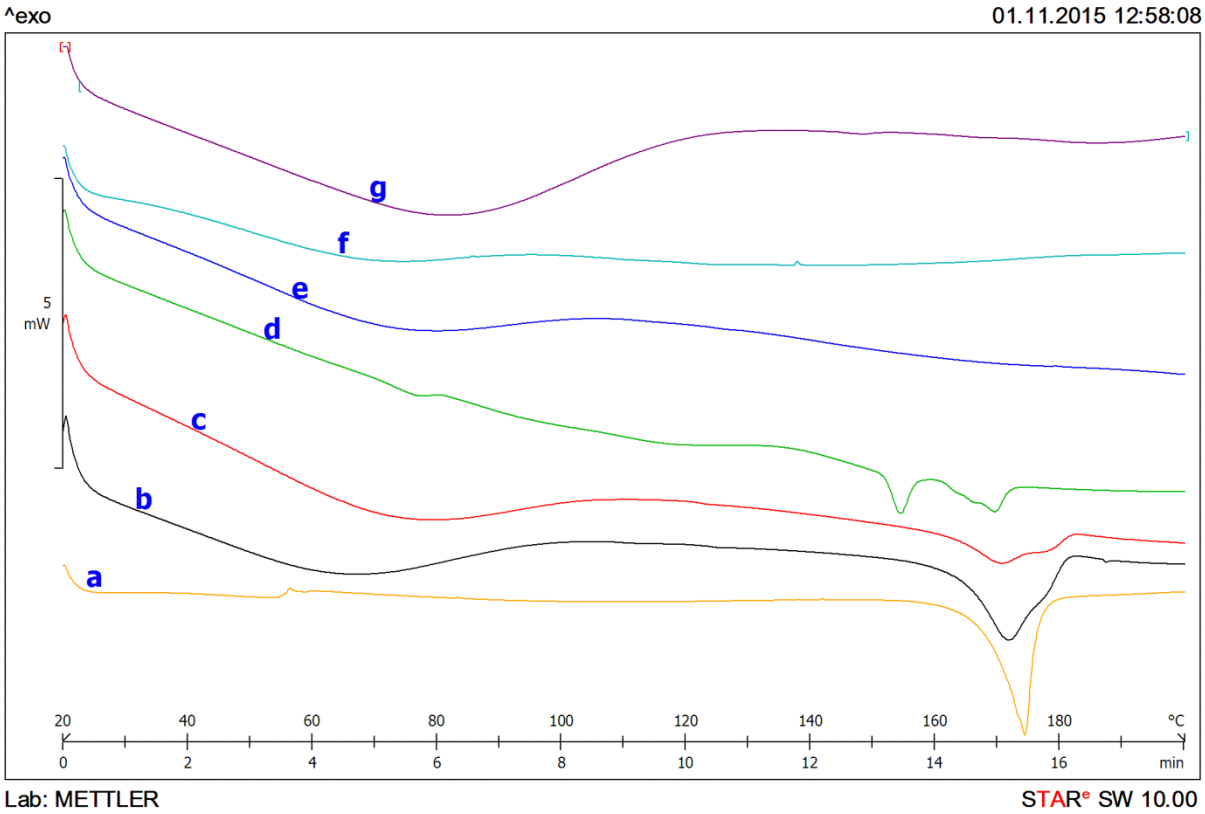

Figure 2. DSC traces of untreated curcumin (a), physical mixtures of curcumin and PVP at ratios of 1:1 (b) and 1:2 (c), curcumin crystallized in absence of PVP (d), curcumin crystallized in the presence of PVP at curcumin: PVP ratios of 1:2 (e), 1:1 (f), and PVP (g).

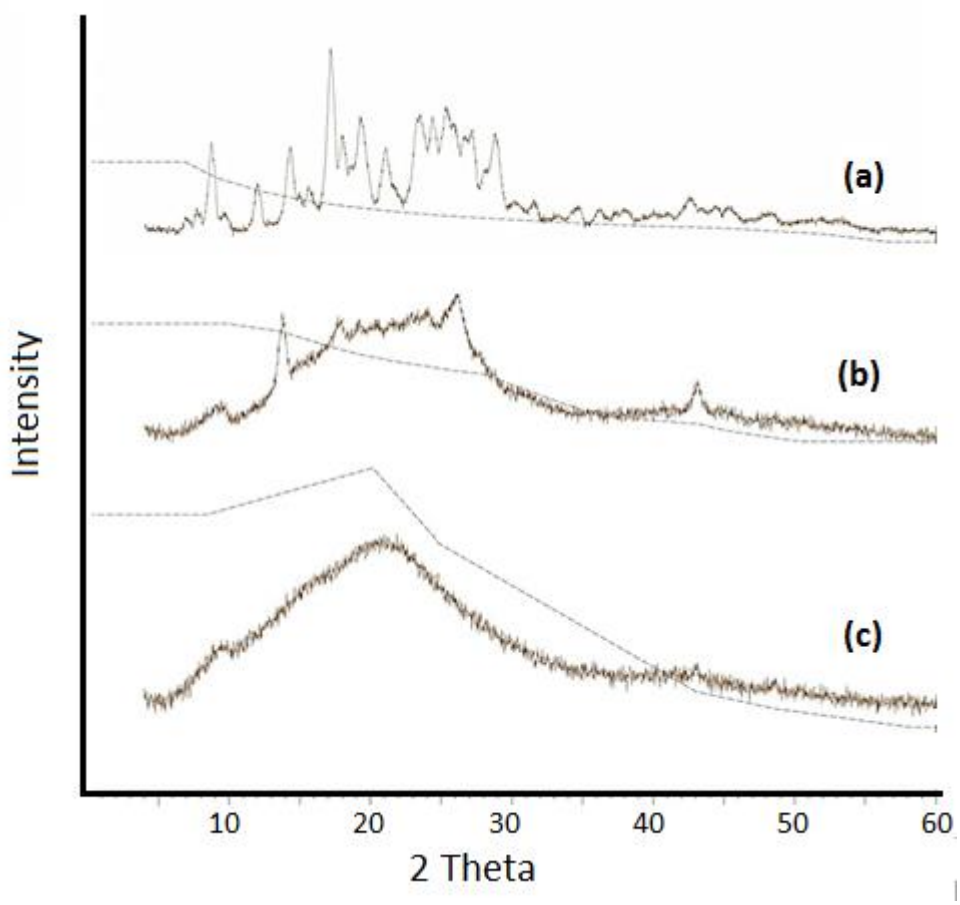

Figure 3. XRPD graphs of untreated curcumin (a), curcumin crystallized in absence of PVP (b), and curcumin crystallized in the presence of PVP at1:1 curcumin:PVP (c).

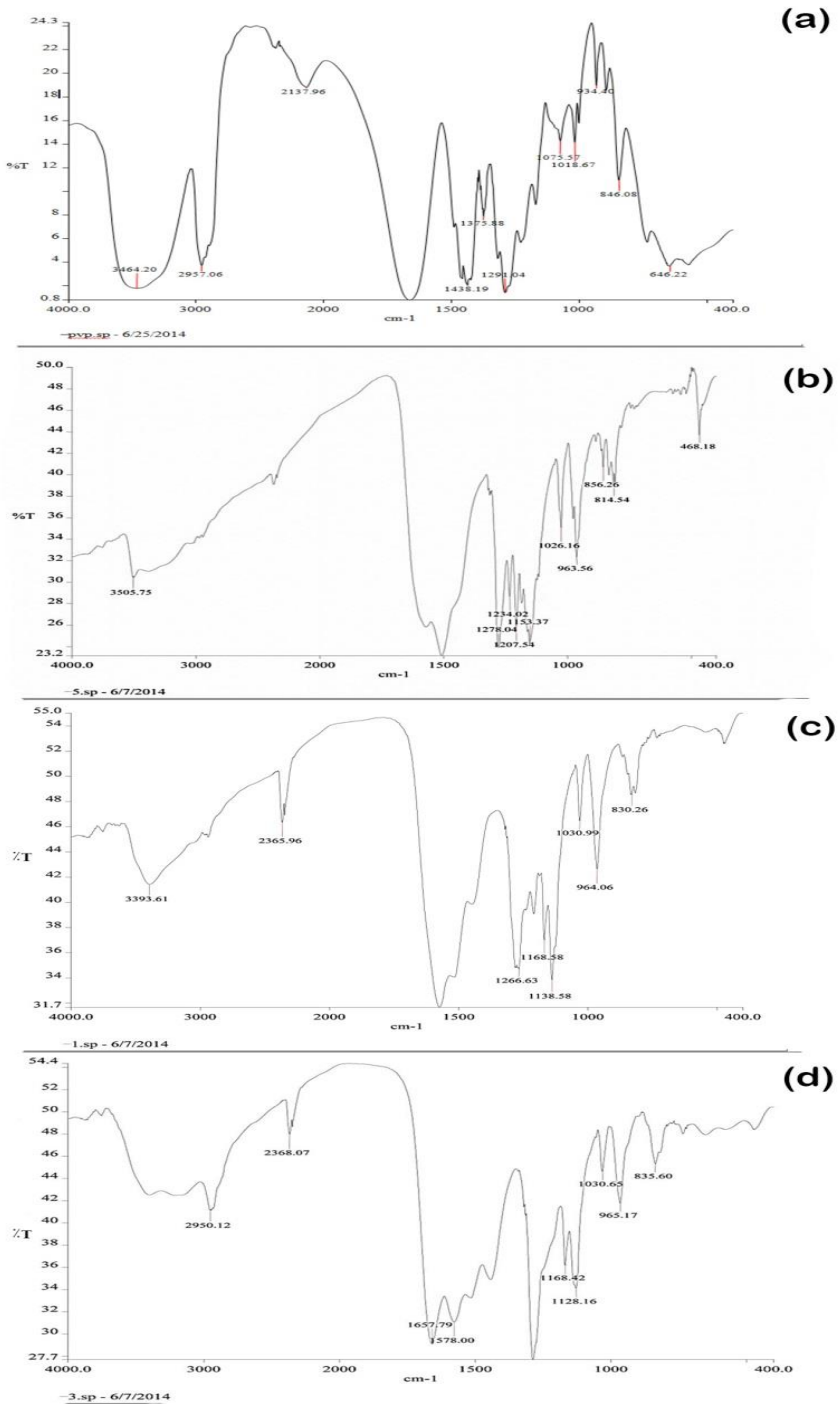

Figure 4. FTIR spectra of PVP (a), untreated curcumin (b), curcumin crystallized in absence of PVP (c), and curcumin crystallized in the presence of PVP at 1:1 curcumin:PVP(d).

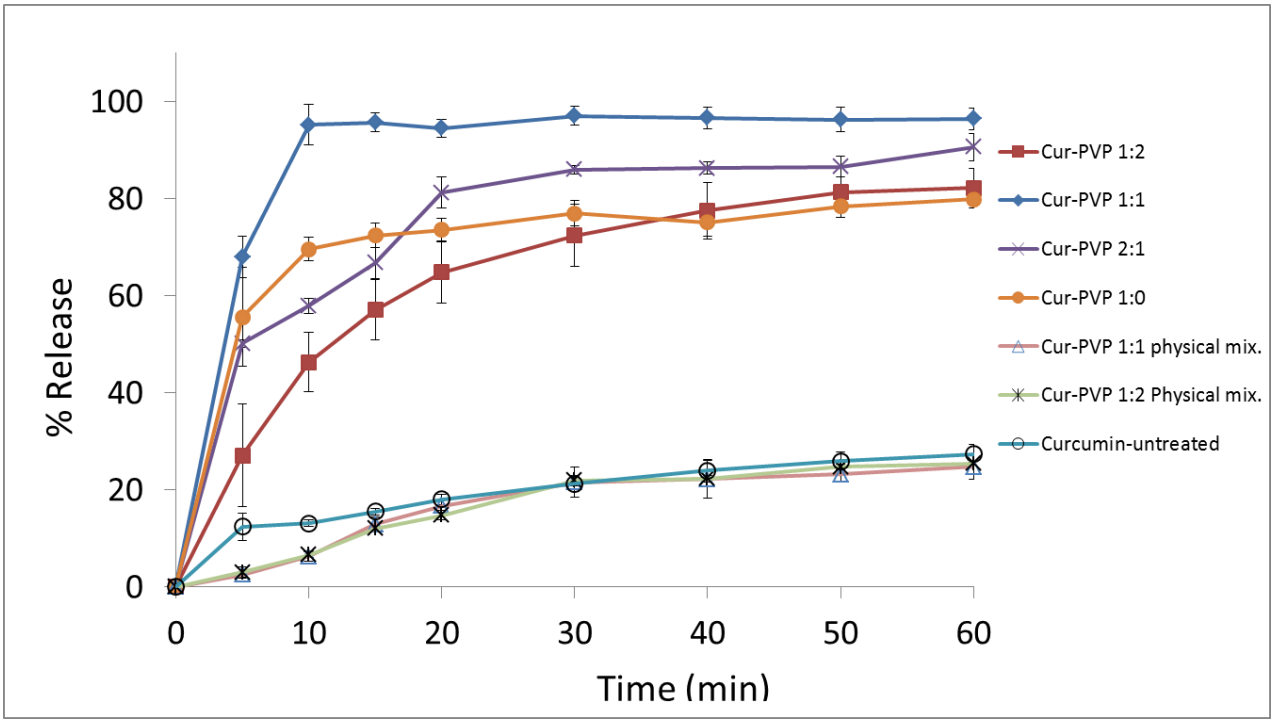

Figure 5. Dissolution profiles of untreated curcumin, physical mixtures of curcumin and PVP, and curcumin samples crystallized in the presence of different amount of PVP **(data are the means and standard deviations of three determinations; n=3).**

Table 1. Solubility of untreated curcumin and different physical mixture and precipitated samples **(data are the means and standard deviation of three determinations; n=3)**

Sample	Solubility (µg/mL)
Untreated curcumin	2.4 ± 0.2
Physical mixture at 1:1 Curcumin:PVP	2.9 ± 0.4
Physical mixture at 1:2 Curcumin:PVP	3.1 ± 0.3
Precipitated sample at 1:0 curcumin:PVP	3.6 ± 0.2
Precipitated sample at 2:1 Curcumin:PVP	3.7 ± 0.2
Precipitated sample at 1:1 Curcumin:PVP	4.6 ± 0.3
Precipitated sample at 1:2 Curcumin:PVP	4.8 ± 0.2